YOUNG INVESTIGATORS AWARDS COMPETITION

408 Young Investigators Awards: Physiology, Pharmacology, and Pathology

Monday, March 18, 2002, 11:00 a.m.-12:15 p.m.
Georgia World Congress Center, Room 257W

11:00 a.m.

408-1 Antirestenotic Effects of Inhibition of Balloon Injury Mediated Apoptosis With Local Delivery of a Caspase Inhibitor

Nirat Beohar, James D. Flaherty, Charles J. Davidson, Lee A. MacDonald, Norman C. Wang, Atman P. Shah, Robert Decker, Jon W. Lumasney, Robert O. Bonow, Francis J. Klocke, *Northwestern Univ Medical School, Chicago, Illinois.*

Background: Restenosis is a major limitation of percutaneous coronary interventions. Barotrauma caused by balloon angioplasty has been shown to trigger early onset of apoptosis in vascular smooth muscle cells (SMC), which may promote migration and proliferation. z-VAD.FMK is a broad spectrum synthetic caspase inhibitor that inhibits apoptosis. **Purpose**: To evaluate if a locally delivered caspase inhibitor, z-VAD.FMK, can protect arterial medial SMCs from balloon injury mediated apoptosis, reducing the subsequent SMC proliferation, thereby limiting restenosis.**Methods**: Bilateral iliac artery angioplasty was performed in 12 male NZW rabbits (Acute = 8; Chronic =4). Simultaneous with balloon injury, the artery was treated locally with normal saline (control) or Z-VAD (contralateral artery). Acute animals were treated with high dose (45,000 ng, n=5) or low dose (4,500 ng, n=3) z-VAD and sacrificed at 4 hours. Apoptosis was detected using TUNEL assay. Apoptotic index was calculated (smooth muscle cell nuclei positive for apoptosis/ 200 smooth muscle cells nuclei counted). In chronic studies, high dose (45,000 ng) z-VAD was delivered locally and animals were sacrificed at 4 weeks. Intimal area (Internal elastic lamina area - Luminal area) and medial area (External elastic lamina thickness - Internal elastic lamina area) were measured. **Results**: The reduction in apoptotic index was 45%, ($p<0.001$) with high dose and 33%, ($p<0.02$) with low dose z-VAD. In the chronic animals, the difference in neointimal area was 39% (4.0 ± 0.6 mm2 vs. 2.4 ± 0.5 mm2) ($p=0.0004$) and in medial area was 20% (7.0 ± 0.7mm2 vs. 5.6 ± 0.4 mm2)($p=0.01$) between control and z-VAD treated arteries.**Conclusions**: Significant inhibition of balloon injury mediated apoptosis of arterial SMCs can be achieved using locally delivered z-VAD, resulting in a significant decrease in both neointimal formation and medial proliferation. This novel antirestenotic strategy is in contradistinction to the conventional approach of causing smooth muscle cell death after onset of cell proliferation.

11:15 a.m.

408-2 Adipose Tissue Remodeling Is Coordinated With Vascular Maturation Through Shifts in Angiopoietin-1 Expression

Susan M. Dallabrida, Joseph Upton, David Zurakowski, Judah Folkman, Karen S. Moulton, Maria A. Rupnick, *Children's Hospital, Boston, Massachusetts, Brigham and Women's Hospital, Boston, Massachusetts.*

Background: Adipose tissue is unique in its plasticity and capacity for vascular remodeling. We hypothesized that these characteristics are enabled by specializations in the maturation state and responsiveness of the adipose vasculature. Adipose tissue vessels, if maintained in an immature state, could be more readily mobilized. If so, a shift toward a stable vasculature may be associated with the loss of adipose tissue pliability that occurs in pathologic fat depots.

Methods: Murine epididymal fat from C57BL6/J and ob/ob mice gaining or losing weight was examined for vascular remodeling molecules. Angiopoietin-1 (ang-1), angiopoietin-2 (ang-2), tie-2, and tie-1 RNA (RT-PCR/ Northern) and protein (Western), and tie-2 phosphorylation were measured (immunoprecipitation/ Western). Adipocyte and endothelial cell specific expressions were determined. Normal human adipose tissue was compared to aberrant fat from arterial (AVM), venous (VM), and lymphatic (LM) vascular malformations, macrodactylies, and lipomas.

Results: Adipose tissue growth and regression were associated with decreased ang-1 mRNA, protein, and tie-2 phosphorylation. Ang-2, tie-2, and tie-1 were stable. Ang-1 inversely correlated with absolute rates of weight change, independent of direction (gained, lost) or etiology (TNP-470, leptin, diet). Adipocytes produced ang-1 and endothelial cells expressed ang-2, tie-2, and tie-1. Ang-2 was 76-99 % lower in aberrant adipose tissue, regardless of source (LM, AVM , macrodactylies, lipomas). PECAM mRNA was similar in normal and affected fat indicating that declines in ang-2 were not due to endothelial loss. Ang-1 increased by 44% in lipomas. Tie-2 increased in AVM (41%) and declined in lipomas (47%).

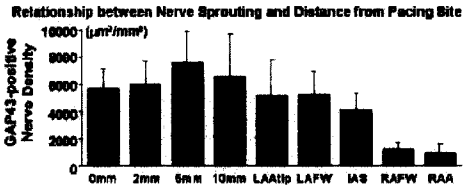
Conclusion: 1) Ang-1 correlates to the rate of change in tissue mass, relating the degree of vessel maturity to the extent of tissue remodeling in normal adipose tissue; 2) Decreased ang-2 in abnormally stable fat suggests a more mature vasculature, which may sustain the affected adipose tissue. Collectively, these findings suggest that specializations in adipose vascular maturation facilitate tissue remodeling, and may offer a point of regulation of adipose tissue mass.

11:30 a.m.

408-3 Induction of Atrial Fibrillation and Nerve Sprouting by Prolonged Left Atrial Pacing in Dogs

Akira Hamabe, Che-Ming Chang, Shengmei Zhou, Yasushi Miyauchi, Yuji Okuyama, Michael C. Fishbein, Hrayr S. Karagueuzian, Lan S. Chen, Peng-Sheng Chen, *Cedars-Sinai Medical Center, Los Angeles, California, UCLA School of Medicine, Los Angeles, California.*

Background: In a canine model of sustained atrial fibrillation (AF) induced by chronic rapid RA pacing, nerve sprouting (NS) is greater in the RA than in the LA. The mechanism is unclear. We hypothesize that NS is induced by electrical current. Therefore, if LA is paced, then NS will be greater in the LA than in the RA **Methods and Results:** Chronic rapid (20 Hz) LA appendage pacing was performed in 5 dogs. Sustained AF (>48 hrs.) was induced within 23±9 days, which was significantly earlier than that with RA pacing using the same protocol (139±84 days). RA, LA and interatrial septum (IAS) were stained with antibodies against growth-associated protein 43 (GAP43) for sprouting nerves and tyrosine hydroxylase (TH) for sympathetic nerves. In all dogs, GAP43-positive-nerve density was highest near the pacing site and decreased with distance from the pacing site. LA and IAS had significantly ($p<0.01$) higher density of GAP43-positive nerves than RA (5723±1579, 4135±1203, 1129±254 mm^2/mm^2, respectively). The TH-positive-nerve density was also highest in LA, followed by IAS and RA (2574±1234, 1487±656, 1007±196 mm^2/mm^2, respectively). The nerves were inhomogeneously distributed within each site, with the greatest heterogeneity observed in LA. **Conclusion:** LA pacing induces sustained AF much faster than RA pacing. In contrast to RA pacing, chronic rapid LA pacing induces greater NS in the LA than in the RA, with the maximum magnitude near the pacing site. These findings indicate that electrical current can induce cardiac NS.



11:45 a.m.

408-4 Acute Neuroprotective Effects of Cortico Steriods Mediated by Nontranscriptional Activation of Endothelial Nitric Oxide Synthase

Florian P. Limbourg, Zhihong Huang, Michael A. Moskowitz, James K. Liao, *Brigham & Women's Hospital, Boston, Massachusetts, Massachusetts General Hospital/Harvard Med. Sch., Boston, Massachusetts.*

Background: Cellular responses to corticosteroids involve the transcriptional modulation of target genes by the glucocorticoid receptor (GR). A rapid, non-transcriptional effect of GR was found to mediate neuroprotection via the activation of endothelial nitric oxide synthase (eNOS).

Methods & Results: In a concentration-dependent manner, dexamethasone stimulated eNOS activity by about 2.5-fold, which was completely inhibited by the GR antagonist, RU486, but not by the transcriptional inhibitor, actinomycin D. Pretreatment with the phosphatidylinositol 3-kinase (PI3K) inhibitor, wortmannin, also inhibited Dex-stimulated eNOS activity, indicating a PI3K-dependent mechanism. Indeed, Dex activated PI3K in a ligand-dependent manner, leading to the phosphorylation and activation of protein kinase Akt and eNOS. In a mouse filament model of transient cerebral ischemia, a bolus injection of Dex (20 mg/kg, i.p.), administered 1 hr before middle cerebral artery occlusion, increased vascular eNOS activity by 2.5-fold, enhanced post-ischemic cerebral blood flow, and decreased cerebral infarct size by 32% (108 ± 9 to 74 ± 8 mm^3, n=11, $P<0.05$). These neuroprotective effects of Dex occurred in the absence of significant changes in physiological parameters and were still evident when Dex was administered 2 hrs *after* ischemia (112 ± 8 to 84 ± 7 mm^3, n=10, $p<0.05$). The beneficial effects of Dex on infarct size were completely absent in $eNOS^{-/-}$ mice, suggesting a novel eNOS-dependent mechanism for stroke protection by corticosteroids.

Conclusion: The non-genomic activation of PI3K/Akt and eNOS by GR represents a physiologically important neuroprotective effect of corticosteroids.

Noon

408-5 The Selective Estrogen Receptor Modulator, Raloxifene, Improves the Severity of Myocardial Ischemia in Canine Hearts

Hisakazu Ogita, Masafumi Kitakaze, Koichi Node, Seiji Takashima, Hiroshi Asanuma, Masanori Asakura, Shoji Sanada, Yoshihiro Asano, Yasunori Shintani, Masatsugu Hori, *Osaka University Graduate School of Medicine, Suita, Japan, National Cardiovascular Center, Suita, Japan.*

Background: We have reported that 17β-estradiol increases coronary blood flow and improves myocardial ischemia. However, little is known as to whether the selective estrogen receptor modulator, raloxifene, mediates coronary vasodilation and improves myocardial ischemia, and what cellular mechanisms are involved in these effects.

Methods: In open-chest anesthetized dogs, the left anterior descending coronary artery (LAD) was perfused through an extracorporeal bypass tube from the left carotid artery. Ral-